REMARKS

ELECTIONS/RESTRICTIONS

Applicants previously provisionally elected Group I, which included claims 1 – 16 and 18, with traverse of the restriction requirement. Applicants acknowledge the Examiner's withdrawal of the restriction requirement for claim 17 and inclusion of claim 17 for examination with elected Group I. Thus, claims 1 – 18 are currently under examination.

REASONS FOR AMENDMENTS

PRIORITY

Applicants request that amendment of page 1, lines 2 – 6, be made to correct the specification in order to comply with the 35 USC §119 requirements for receiving benefit of the earlier filing date of provisional application 60/434,242 filed on 18 December 2002, priority to which was claimed in Applicants' declaration for the present application.

This amendment does not add new material to the application.

SPECIFICATION

Applicants also request amendment of page 26, lines 22 – 23, in order to correct the typographic error pointed out by the Examiner.

This amendment does not add new material to the application.

CLAIMS

Applicants request that amendment of claims 1, 3, 5, 6, 7, 8, 11, 12, 13, 14, 16, and 18 be made. The reasons for these amendments are discussed below. None of these amendments add new material to the application.

OBJECTIONS (INFORMALITIES)

Amendment of page 1, line 1, and of page 26, lines 22 – 23, as requested above by Applicants obviates the objections to the specification made by the Examiner. Applicants respectfully request that the objections be withdrawn.

REJECTIONS

CLAIMS ARE DEFINITE UNDER 35 USC §112, SECOND PARAGRAPH

The Examiner has rejected numerous claims as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Applicants respectfully contend that the claims as originally filed are definite under 35 USC §112, second paragraph. To expedite allowance of the application, however, Applicants submit this amendment to clarify the claimed invention.

The Examiner has rejected claim 5 for alleged indefiniteness of the phrase "plant physiological benefit". Claim 5 has been amended to more clearly describe the attributes conferred on the claimed transgenic plant. Support for the amendment is found, for example, on page 4, lines 5 – 15:

> Potentially any exogenous DNA can be operably linked to the embryo-specific promoter, including, for example, a selected sequence which encodes a molecule imparting insect resistance, bacterial disease resistance, fungal disease resistance, viral disease resistance, nematode disease resistance, herbicide resistance, enhanced grain composition or quality, nutrient transporter functions, enhanced nutrient utilization, enhanced environmental stress tolerance, reduced mycotoxin contamination, female sterility, a selectable marker phenotype, a screenable marker phenotype, a negative selectable marker phenotype, altered plant agronomic characteristics, or a combination thereof. In preferred aspects of this invention, the embryo-specific promoter is operably linked to heterologous DNA which encodes a molecule imparting enhanced kernel development, embryo development, general production or protection of next-generation tissues, or grain agronomic characteristics.

Additional support for amended claim 5 can be found, for example, on page 12, lines 18 - 26:

> Such traits include, but are not limited to, herbicide resistance, herbicide tolerance, insect resistance, insect tolerance, disease resistance, disease tolerance (viral, bacterial, fungal, nematode), stress tolerance, stress resistance, as exemplified by resistance or tolerance to drought, heat, chilling, freezing, excessive moisture, salt stress and oxidative stress, increased yield, food content and value, increased feed content and value, physical appearance, male sterility, female sterility, drydown, standability, prolificacy, starch quantity and quality, oil quantity and quality, protein quality and quantity, amino acid composition, and the like. It is also anticipated that expression of exogenous DNA encoding antisense RNAs or other RNA molecules are included as useful means for modifying plant phenotype.

Yet more support for amended claim 5 can be found in the specification under the heading "5. Exogenous Genes for Modification of Plant Phenotypes", which includes numerous examples of attributes that can be conferred on the claimed transgenic plant, as was known in the art at the time the application was made, and were incorporated by reference.

Claim 6 has been rejected by the Examiner for indefiniteness of the recitation "RNA imparting gene suppression". Applicants present amended claim 6 to more clearly claim the invention. Support for the amendment may be found, for example, throughout the specification text under the heading "5. Exogenous Genes for Modification of Plant Phenotypes" (see page 14, line 13, to page 16, line 23), which includes numerous examples of RNA-mediated gene suppression as was known in the art at the time the application was made and was incorporated by reference. Applicants further present amended claim 7, which amendment more clearly defines the invention.

The Examiner has rejected claim 8 for the alleged indefinite use of the word "if". Amended claim 8 omits this language, rendering the rejection moot.

Claims 11, 12, and 13 have been amended as the Examiner has helpfully suggested, to more clearly point out that the claimed seeds contain the claimed exogenous DNA construct.

Applicants have amended claim 14 to more clearly define the invention. Support for the amendment can be found throughout the specification, for example, on page 2, lines 23 – 25:

> One aspect of the invention provides a DNA construct comprising a promoter operably linked to an exogenous DNA, where the promoter is derived from part or all of the 5' regulatory region of a maize *emb5* gene and exhibits promoter activity.

The Examiner has rejected claim 18 for being indefinite in its recitation of a "substantially purified" DNA. Applicants have amended the claim for clarity and respectfully submit that isolating or purifying DNA to a given degree is routine in the art, and that one skilled in the art would be familiar with the appropriate degree of isolation or purification to which DNA should be prepared for various purposes, as is shown in the numerous cited publications which were incorporated by reference into the specification. The working examples included in the specification (see, for example, page 24, line 17, through page 27, line 18) further demonstrate that Applicants were certainly in possession of the knowledge and skills to isolate or purify a DNA of the invention as claimed.

The Examiner has alleged that the recitation "*emb5*" in claims 1 and 17 renders these claims indefinite. Applicants respectfully disagree. Applicants additionally note that the recitation "*emb5*" does not appear in claim 17, and assume that the Examiner intended to refer to claim 14, from which claim 17 depends.

Applicants maintain that the recitation "*emb5*" is clearly described in the specification as filed. For example, page 8, lines 10 – 16, states:

> EMB5 is a late embryogenesis-abundant protein and the *emb5* gene is abscisic-acid responsive and expressed in maize embryos. Williams and Tsang (*Dev. Genetics*, 15:415-424, 1994) reported that *emb5* transcripts are first detected during mid-embryogenesis (approximately 21 days after pollination (DAP)) and accumulate to peak levels by late embryogenesis (about 27 DAP). RNA levels gradually decrease to very low levels as the seed matures to approximately 48 DAP. *Emb5* transcripts show greatly reduced levels in kernels following imbibition and are not found in seedlings.

Page 24, lines 17 – 22, further states:

> The 5' regulatory region of a maize embryo-specific *emb5* gene was isolated from genomic DNA of a public maize line Missouri 17 (MO17). Oligonucleotide primers of SEQ ID NO:2 and SEQ ID NO:3 for the PCR reaction were designed based upon a public protein sequence for maize *emb5* (Williams and Tsang, *Plant Physiol.*, 100:1067-1068, 1992; MZEEMBIV; accession number M90554) and were used to PCR amplify the a portion of the emb5 promoter from MO17 genomic DNA.

In conjunction with the explicit support for the recitation "*emb5*", a promoter derived from the 5' regulatory region of an *emb5* gene, and exhibiting promoter activity in plants, is clearly described by the specification as filed, for example, on page 2, lines 23 – 25:

> One aspect of the invention provides a DNA construct comprising a promoter operably linked to an exogenous DNA, where the promoter is derived from part or all of the 5' regulatory region of a maize *emb5* gene and exhibits promoter activity.

Further support is found, for example, on page 6, lines 12 – 22:

> Promoters are located upstream of the coding sequence to be transcribed and have regions that act as binding sites for RNA polymerase and have regions that work with other factors to promote RNA transcription. More specifically, basal promoters in plants comprise canonical regions associated with the initiation of transcription, such as CAAT and TATA boxes. The TATA box element is usually located approximately 20 to 35 nucleotides upstream of the site of initiation of transcription. The CAAT box element is usually located approximately 40 to 200 nucleotides upstream of the start site of transcription. The location of these basal promoter elements result in the synthesis of an RNA transcript comprising nucleotides upstream of the translational ATG start site. The region of RNA upstream of the ATG is commonly referred to as a 5' untranslated region or 5' UTR.

Yet more support is found on page 6, lines 26 – 27:

> As used herein "promoter activity" characterizes a DNA sequence which initiates transcription of RNA from adjacent downstream DNA.

Page 8, lines 24 – 27, provides further literal support for the claim:

> An *emb5* promoter sequence of the invention is exemplified by the nucleic acid sequence given in SEQ ID NO:1. Characterization of the nucleic acid sequence of SEQ ID NO:1 indicates that transcription initiation begins at about nucleotide 1553. Canonical TATA and CAAT boxes are identified as beginning at about nucleotides 1505 and 1411, respectively, in SEQ ID NO:1.

Applicants maintain that the claims as originally filed are definite under 35 USC §112, second paragraph. In view of the amendments made herein nonetheless for clarity, Applicants submit that the Examiner's rejections are made moot, and respectfully request that the rejections be withdrawn.

CLAIMS COMPLY WITH BOTH THE WRITTEN DESCRIPTION REQUIREMENT AND THE ENABLEMENT REQUIREMENT UNDER 35 USC §112, FIRST PARAGRAPH

The Examiner has rejected claims 1, 3 – 14, and 16 – 18, under 35 USC §112, first paragraph, as failing to comply with the written description requirement. The Examiner has further rejected claims 1, 3 – 14, and 16 – 18, under 35 USC §112, first paragraph, as failing to comply with the enablement requirement. Applicants respectfully disagree, and wish to direct the Examiner to the Board of Patent Appeals and Interferences' Opinion in support of the Decision on Appeal under 35 USC §134 *ex parte* McElroy (United States Patent Application No. 09/532,806, now United States Patent Number 6,747,189, issued 8 June 2004), which is part of the public record and contains relevant discussions of compliance with 35 USC §112, first paragraph. A copy of this Opinion is attached herein for the Examiner's convenience.

Applicants maintain that claims 1, 3 – 14, and 16 – 18 comply with both the written description requirement and with the enablement requirement of 35 USC §112, first paragraph. Specifically, applicants maintain that claims 1, 3, 14, 16, and 18 comply with both the written description requirement and with the enablement requirement as originally filed. Nonetheless, in the interest of expediting examination, claims 1, 3, 14, 16, and 18 have been amended to more clearly define the invention. Claims 1, 14, and 18 now recite that the claimed promoter exhibits embryo-specific activity in plants. Claims 3, 16, and 18 now recite that the claimed about 100 to

about 1650 contiguous nucleotides of DNA have from 85% to 100% sequence identity to at least one segment of SEQ ID NO: 1. The claims are fully supported by the specification as originally filed.

The specification as originally filed literally and fully describes a promoter derived from the 5' regulatory region of an *emb5* gene, having the sequence of SEQ ID NO: 1, and exhibiting promoter activity in plants. For example, Figure 1 of the specification describes SEQ ID NO: 1 by naming its 1658 contiguous nucleotides. In doing so, the specification *prima facie* describes each and every promoter comprising from about 100 to about 1650 contiguous nucleotides of SEQ ID NO: 1. Applicants submit that it would be immediately clear to one skilled in the art what is claimed as an aspect of the Applicants' invention, that is, each and every nucleotide sequence comprising from about 100 to about 1650 contiguous nucleotides of SEQ ID NO: 1 and having embryo-specific promoter activity in plants is claimed.

The Examiner alleges that the specification does not describe how the sequence of SEQ ID NO: 1 can be changed without altering its abscisic acid-inducible, embryo-specific transcriptional activity, that the specification does not describe regions of SEQ ID NO: 1 that are essential to its activity, and generally alleges that the specification does not describe derivative promoters comprising from about 100 to about 1650 contiguous nucleotides and having between about 85% to about 100% sequence identity to SEQ ID NO: 1. Applicants respectfully disagree. Support for identifying derivative promoters, which exhibit promoter activity similar or identical to that of the maize *emb5* promoter, is profuse within the specification as filed. Such derivative promoters may include nucleotide sequences that are fragments of SEQ ID NO: 1, or that are derived from SEQ ID NO: 1, as clearly described in the specification. Such derivative promoters may further include other sequences (such as heterologous CAAT or TATA boxes or other transcriptional start site sequences), literal support for which is given in the specification. See, for example, page 9, line 5, to page 10, line 11 (emphases added):

> This invention provides derivatives of the embryo-specific promoter which has been derived from the 5' regulatory region of the maize *emb5* gene. **Derivatives of this promoter may include, but are not limited to, deletions of sequence, single or multiple point mutations, alterations at a particular restriction enzyme site, addition of functional elements, or other**

means of molecular modification which may enhance, or otherwise alter promoter expression. For example, one of skill in the art may delimit the functional elements within the *emb5* promoter and delete any non-essential elements. Functional elements may be modified or combined to increase the utility or expression of the sequences of the invention for any particular application. For example, a functional region within the *emb5* promoter of this invention can be modified to decrease or increase inducible expression. The means for mutagenizing or creating deletions in a DNA segment encoding any promoter sequence, such as an *emb5* promoter sequence of the current invention, are well known to those of skill in the art and are disclosed, for example, in U.S. Patent No. 6,583,338, incorporated herein by reference in its entirety.

It is anticipated that fragments of the *emb5* promoter that are especially useful for allowing functionality include, but are not limited to, the about 100 nucleotide base pair 5' UTR region from the transcriptional start site to the ATG (about nucleotide 1553 to 1658 of SEQ ID NO:1), the about 250 nucleotide base pair 5' UTR region from the CAAT box to the ATG, including the TATA box (about nucleotide 1411 to about 1658 of SEQ ID NO:1), the about 140 nucleotide base pair 5' UTR region from the CAAT box to the transcriptional start site (from about nucleotide 1411 to about 1553 of SEQ ID NO:1), and the about 1000 or more nucleotide base pair 5' UTR region upstream of the CAAT box (from about nucleotide 1 to about 1411 of SEQ ID NO:1), and fragments thereof. The various fragments described may be operably joined to a heterologous DNA and used for plant transformation. Exogenous DNA comprising a marker gene or reporter gene is useful for testing the promoter activity of the various fragments. It is also anticipated that the sequences and fragments thereof upstream of the CAAT box (from about nucleotide 1 to about 1411 of SEQ ID NO:1) may be operably linked to heterologous CAAT and TATA boxes or other transcriptional start site sequences and exhibit promoter activity similar or identical to that of the maize *emb5* promoter.

Thus, promoters of this invention are not required to have 100% sequence identity to SEQ ID NO:1. For instance, effective promoters can range from about 85% to about 100% identity to SEQ ID NO:1 or a fragment of SEQ ID NO:1, e.g. a DNA fragment of at least about 100 nucleotide base pairs, or larger, e.g., about 150, about 250 or about 100 nucleotide base pairs. In one aspect of the invention the promoters and derivative promoters are characterized as having at least about 85% sequence identity, more preferably at least about 90% sequence identity or higher, e.g., at least about 95% or even about 98% sequence identity with SEQ ID NO:1, or such a fragment thereof.

The Examiner further states that no promoter is described that has any sequence identity with nucleotides 1554 – 1658 of SEQ ID NO: 1. Applicants point out that SEQ ID NO: 1, which is literally and precisely described in structure and function, includes these nucleotides. Persons skilled in the art would immediately recognize that derivative promoters can clearly include sequences derived from nucleotides 1554 – 1658 of SEQ ID NO: 1, and that such derivative promoters could be assayed for promoter activity by the various techniques disclosed by the specification.

The Examiner states that the specification does not describe any other *emb5* gene, or the function of maize *emb5*, and thus does not enable one skilled in the art to identify other *emb5* genes, and corresponding promoters, from maize or any other plant. Applicants respectfully disagree. Applicants' claims are directed to *emb5* promoters, and not to an *emb5* gene nor to an *emb5* gene's function. The specification explicitly describes a method for cloning the maize *emb5* promoter. See page 24, lines 17 – 27:

> This example discloses a method for cloning the maize *emb5* promoter. The 5' regulatory region of a maize embryo-specific *emb5* gene was isolated from genomic DNA of a public maize line Missouri 17 (MO17). Oligonucleotide primers of SEQ ID NO:2 and SEQ ID NO:3 for the PCR reaction were designed based upon a public protein sequence for maize *emb5* (Williams and Tsang, *Plant Physiol.*, 100:1067-1068, 1992; MZEEMBIV; accession number M90554) and were used to PCR amplify the a portion of the emb5 promoter from MO17 genomic DNA. The cloned fragment was used as a probe against maize MO17 genomic DNA lambda FIX II from which a genomic clone containing additional emb5 promoter sequence was isolated. Addition PCR using PCR primers EMPRO-433 (SEQ ID NO:4) and EMPRO-1 (SEQ ID NO:5) and standard cloning procedures were used to clone the emb5 promoter having the sequence of SEQ ID NO:1 contained in a vector designated pEMpro.

That the specification is fully enabling of the claimed invention is incontestable as the resulting identified *emb5* promoter was shown to have promoter activity in plant embryos (see Tables 1 and 2 of the specification).

Furthermore, Applicants respectfully remind the Examiner that it is exceedingly well known and routinely practiced in the art to search for sequences that are homologous to a given gene, and that such a search for and identification of homologous sequences does not require any knowledge of the given gene's function. It would be immediately apparent to one skilled in the art to search, for example, for other maize or non-maize sequences homologous to the maize *emb5* gene, and to clone the promoters of such identified *emb5* homologues, or, alternatively, to search for other maize or non-maize sequences homologous to the maize *emb5* promoter, or homologous to SEQ ID NO: 1. See, for example, page 10, lines 12 -14:

> Promoters of this invention can also be derived from homologous genes from species other than maize. For example, promoters of this invention can be derived from the 5' regulatory region of a rice or wheat gene homologous to the maize *emb5* gene.

The specification further describes methods for detecting promoter activity in plant embryos, including transgenic plants, and fully enables methods for detecting promoter activity of any derivative promoter of the invention (such as derivatives obtained from homologous sequences). See Examples 2, 3, 4, and 5 of the specification, which explicitly describes detection of promoter activity and especially embryo-specific promoter activity, by use of a reporter gene (*uidA* or GUS) operably linked to a promoter of the invention. See page 9, line 26, to page 8, line 3:

> The various fragments described may be operably joined to a heterologous DNA and used for plant transformation. Exogenous DNA comprising a marker gene or reporter gene is useful for testing the promoter activity of the various fragments. It is also anticipated that the sequences and fragments thereof upstream of the CAAT box (from about nucleotide 1 to about 1411 of SEQ ID NO:1) may be operably linked to heterologous CAAT and TATA boxes or other transcriptional start site sequences and exhibit promoter activity similar or identical to that of the maize *emb5* promoter.

Many other techniques for assaying promoter activity are routine in the art and disclosed in the specification. See page 17, line 25, to page 18, line 6:

> To confirm the presence of an exogenous DNA in regenerated plants, a variety of assays may be performed. Such assays include, for example, molecular biological assays such as Southern and Northern blotting and PCR; biochemical assays such as detecting the presence of a protein product, *e.g.*, by immunological means (ELISAs and Western blots) or by enzymatic

function; plant part assays such as leaf or root assays; and in some cases phenotype analysis of a whole regenerated plant. Additional assays useful for determining the efficiency of transgene expression and promoter function also include without limitation fluorescent *in situ* hybridization (FISH), direct DNA sequencing, pulsed field gel electrophoresis (PFGE) analysis, single-stranded conformation analysis (SSCA), RNase protection assay, allele-specific oligonucleotide (ASO), dot blot analysis, denaturing gradient gel electrophoresis, RT-PCR, quantitative RT-PCR, RFLP and PCR-SSCP. Such assays are known to those of skill in the art.

See even further page 20, lines 6 – 9:

Screenable markers which provide an ability to visually identify transformants can also be employed, *e.g.*, a gene expressing a colored or fluorescent protein such as a luciferase or green fluorescent protein (GFP) or a gene expressing a *beta*-glucuronidase or *uidA* gene (GUS) for which various chromogenic substrates are known.

Applicants finally maintain that it is well known in the art to introduce a transgene (such as a promoter and operably linked gene) into a plant cell or a plant, as is disclosed in detail by the specification and the incorporated references therein. Applicants acknowledge the need for experimentation, but firmly maintain that such need is recognized as routine and would not be considered "undue" by one skilled in the art. The working examples and further guidance present in the specification fully enable one of ordinary skill in the art to provide a transgenic plant as presently claimed.

Applicants maintain that the claims as originally filed comply with both the written description requirement and with the enablement requirement of 35 USC §112, first paragraph. In view of the amendments made herein nonetheless for clarity, Applicants believe that the Examiner's rejections are made moot, and respectfully request that the rejections be withdrawn. Applicants respectfully submit that the claims are ready for examination and in condition for allowance.

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If the Examiner has any questions regarding this application, the Examiner is encouraged to contact Applicants' undersigned agent at (860) 572-5217 (telephone) or (860) 572-5280 (fax).

Respectfully submitted,

Maria Margarita D. Unson
Registration Number 53,711
Agent for Applicants

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